THE EFFECTS OF THE LATHYROGENIC AGENT, PROPIONITRILE, ON THE VERTEBRAL AXIS OF CHICK EMBRYOS

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CHAPTER I

INTRODUCTION

Lathyrism, a syndrome that has been described since the time of Hippocrates, was reported in medical journals to be a common disease in central and northwestern parts of India as early as 1868. This syndrome, caused by the consumption of Lathyrus odoratus (the flowering sweet pea) in the diet, was characterized by lameness, rigidity of the leg muscles, spinal curvature and affected the connective tissues of the body.

Experimental lathyrism has become a means of investigating the diseases of connective tissue, since the symptoms of lathyrism were characterized by a weakness of the fibrous tissue and disordered growth of cartilage and bone. When the mechanism of the actions of lathyrogens is understood, it may be an important facet in the study of the formation and degeneration of connective tissue.

The aim of this investigation was to determine the effects of the lathyrogenic agent, propionitrile, on the development of the connective tissues of the vertebral axis during chick embryogenesis. Data from such a study would be meaningful in determining specific targets of the lathyrogen in a developing system.

CHAPTER II

REVIEW OF LITERATURE

The embryological development of the vertebral column in the chick has been investigated extensively for many years. Studies on the development of the vertebral column led to an investigation of sclerotome formation, since the sheath around the notochord and other supporting tissue was found in some instances to come from sclerotomal cells. According to Gadow (1933), the first comprehensive study of the sclerotomes and their derivatives was presented by Remak in 1855. Remak found that chick vertebrae were formed by a recombination of parts of adjacent sclerotomes. Subsequently, von Ebner supported Remak's view by discovering a transitory vertical fissure dividing the sclerotomes of the chick into approximately equal halves.

Gadow also stated that in 1928, Piiper proposed certain ontogenetic and phylogenetic stages in the development of the vertebral column. Piiper distinguished four main stages in the membranous and cartilaginous condition of the column: (1) the form of the notochord, (2) absence or presence of the perichordal and vertebral rings, (3) the intrasclerotomal fissure, and (4) the intercentra.

Williams (1942) did an extensive study on the development of chick vertebrae under normal and experimental conditions. A study of normal conditions showed that the development of the cervical somites of a 48-hour chick embryo was differentiated into dorsolateral dermato-myotome and into ventro-medial sclerotome. In the anterior cervical region the mesenchymal cells migrated into the region lateral to the notochord and into the space between the notochord and entoderm. These mesenchymal cells connected with

each other to form a syncytial tissue. The processes of these cells also extended to the notochord where they gave rise to its fibrous sheath. This condition was clear in the region of the first somite, where the fibrous sheath was distinguishable. By the 55th hour of incubation, each sclerotome was divided into equal anterior and posterior halves by a region sparse in cells. In the 85-hour chick embryo it was possible to see a difference in the disposition of the cells in the dorsal and ventral parts of the caudal sclerotome. The cells of the dorsal part were radially arranged with respect to the notochordal sheath, whereas those of the lower portion showed no definite arrangement.

In connection with the origin of the fibrous notochordal sheath, the mesenchymal cells from the sclerotome became closely applied to the surface of the notochord at an early stage of development. It was pointed out that wherever mesenchyme existed, the possibility for connective tissue development also existed. Between the 60th and 80th hours of incubation these mesenchymal cells increased in number and flattened out to form a continuous covering, the cellular perichordal sheath. By the 85th hour of incubation the perichordal sheath differentiated. The inter-sclerotomic formation of the perichordal tube consisted of sparse cells, which were arranged as a vertebral ring. In a five-day-old chick embryo, the vertebral ring became pre-cartilaginous. Its spindle-shaped cells were arranged concentrically around the notochord. The six-day-old embryo was only slightly different from the preceding stage. The primary and secondary vertebral bodies were now cartilaginous. By the seventh day of incubation the fundamental plan of the vertebra was established.

An experimental analysis of the development of the vertebral column was accomplished using the transplantation method. Williams (1942) transplanted the notochord, perichordal mesenchyme, perichordal rings, early centra and somites onto the chorio-allantoic membrane during embryogenesis. From this study of normal and experimental development of the vertebral column, he concluded that the sclerotomes were divided into cranial and caudal parts (called sclerotomites) by the intra-sclerotomic fissure. Mid-sclerotomic dilatations of the 85-hour-old notochord in the chick embryo became enclosed by a ring-like formation, the perichordal ring, which later fused to form a continuous perichordal tube. The primary vertebral body represented an intersclerotomic differentiation of the perichordal tube. The notochord was found to play only a mechanical role in the formation of the centra. Normal and experimental evidence indicated that the fibrous notochordal sheath was not a product of the outer notochordal cells, but was formed from surrounding mesenchymal cells.

The results of the experimental study by Williams reported evidence for the suggestion of Feller and Sternberg that there were two parts to the development of the vertebral column: (1) arches, depending on the central nervous system; (2) centra, depending on the notochord. Williams' findings that the notochordal sheath of the chick was formed from the processes of the surrounding mesenchymal cells were corroborated by the view of Studnicka, who first suggested that the sheath originated from the adjacent tissue. Williams reported that Kuhlenbeck came to the same conclusion after making a critical analysis of notochordal sheath development in the duck.

Experimental alteration in the normal development of the vertebral

column was found to be a characteristic feature of the syndrome of lathyrism. Early investigators related that lathyrism was found to be a common disease in certain parts of India and in scattered regions of Europe. In 1929, Ralph Stockman reported that in certain parts of India where peas (Lathyrus sativus) were used extensively in the diet, lathyrism resulted due to a poison which was isolated in an impure form from the pea plant. When consumed in moderate amounts, the peas were found to be a pleasant and nourishing food; however, epidemics resulted during times of famine due to an increase in consumption. At this time, lathyrism was found to affect the motor nerves of man and the respiratory tract of horses, and to react varyingly in different species in reference to susceptibility and pathological effects. A few years later, Geiger, Steenbock and Parsons (1933) investigated lathyrism in the rat and found that it produced lameness, spinal curvature and malformation of long bones. The toxic factor which produced lathyrism was found to be extractable from the flowering sweet pea by boiling water. The lathyrogenic chemicals studied in all early investigations, both in man and rats, were found to produce paralysis as well as deformation of the lower extremities.

The effects of the lathyrogenic agents have been investigated by various biochemical and histological indices. Chang, Witschi and Ponseti (1955) studied the teratogenic effects of Lathyrus odoratus seeds on the limbs of chick embryos and found that the chemical beta-aminopropionitrile (BAPN) was the agent which produced bending of the femurs. Bending occurred in the primary ossification center on the thirteenth day of incubation; however, cartilage and bone were histologically normal. The available evidence from

this study suggested that a particular process essential in development was interrupted by the presence of the BAPN during differentiation.

Although it had been demonstrated by Bachhuber, Lalich, Angevine, Schilling and Strong (1959) that beta-aminopropionitrile was the natural causative agent of lathyrism, structurally related agents were also found to produce similar results. Neuman, Maxwell and McCoy (1956) found that structurally related semicarbazide hydrochloride (SCH) produced some characteristic inhibition of development of chick embryos. Following injections of SCH (aqueous solution) into the yolk of 4-day-old embryos, they reported skeletal malformations in the beak and tibiotarsus. The best results of bone deformation were obtained when injections were made between the fourth and sixth day. Injections made earlier than four days proved to be less effective. Their findings represented the first account of lathyrism in chick embryos produced by the injection of semicarbazide hydrochloride into the yolk.

Experiments reported by Follis and Tousimis (1958) indicated that the developmental processes affected by lathyrogens, referred to in earlier studies by Chang, Witschi and Ponseti (1955) were collagen fibers. In the investigation by Follis and Tousimis, it was found that rats treated with BAPN and aminoacetonitrile (AAN) exhibited profound physical and structural changes in the epiphyseal cartilage. Electron microscopic studies of the cartilage from the treated rats showed a marked reduction in the number of collagen fibers. Gardner (1958) studied the production of bone lathyrism, using Lathyrus odoratus and beta-substituted ethylamines. He reported the appearance of altered chondrocytes in the developing cartilage, leading to

an irregular development and deformation in the shape of the new cartilage in the rat skull. The deformation process, however, was not uniform. These findings by Gardner led to the postulation that all disorders described in lathyrogenic studies had a common pathogenesis. Later studies by Gardner (1960), using oral connective tissue, provided histochemical evidence which suggested that the homogeneous substance deposited in ligaments during experimental lathyrism was altered collagen. The connective tissue was of a nature intermediate between typical collagen and hyaline non-fibrous collagen. These studies led to the conclusion that lathyrism represented either an alteration in the structure of collagen fibers, or the formation of an organic-soluble type collagen. This work aided in confirming evidence presented by Follis and Tousimis that the action of lathyrogenic agents was on the collagen fibers.

Dasler (1958) contributed to the study of lathyrism when he found that SCH, injected into the yolk sacs of incubating eggs, affected the skeleton in a way similar to the action of BAPN. The finding that semicarbazide hydrochloride produced lathyritic skeletal lesions was important because it demonstrated that the presence of a nitrile group was not essential for osteolathyritic activity. This gave a new group of lathyrogenic compounds, since the most prominent heretofore had been BAPN and AAN.

Van den Hooff, Levene and Gross (1959) studied collagen changes in chick embryos treated with BAPN. Theirs was an electron microscopic and histological study on the skin of normal and lathyritic chick embryos leading to information on the state of the lathyrogenic collagen in the tissue. Histological evidence obtained from this study by lathyritic animals

indicated that dermal fibers were entirely absent in some areas, while vestiges were found in places. In other regions large lakes of amorphous collagen-staining material appeared. The "reticulin" fibrils were found to be diminished in number. Comparison of this study with the work of Follis and Tousimis (1958) was found to be contradictory. Van den Hooff, Levene and Gross (1959) revealed that collagen present in lathyritic tissue was in fibrillar form; Follis and Tousimis reported that new collagen was being synthesized but not organized into fibrillar form, and it remained dispersed. Levene and Gross (1959) found that when BAPN and SCH injections were made into the choric-allantoic membrane of chick embryos after fourteen days of incubation, there was an increase in extractable collagen. They concluded that at least one of the defects induced by lathyrogenic agents was an interference with the normal intermolecular cross-linking within the collagen fibril, thereby agreeing with the work of Van den Hooff, Levene and Gross.

Later work by Levene, Gross and Orloff (1960), using chick embryos injected with BAPN and SCH on the fourteenth day of incubation, reported a loosening of the intermolecular structure within the collagen fibrils and a marked increase in fragility of the entire animal. Definite effects were produced in the skin, bone, aorta and tendons of the lathyritic animal. This work agreed with earlier studies by Van den Hooff, Levene and Gross (1959). Enzinger and Warner (1960) studied the effect of AAN on the histology of connective tissue growth. The more striking lesions showed degeneration of cartilaginous matrix, proliferation of cartilage cells, and detachment of ligaments. Collagen formation was reported unaltered in the

lathyritic cartilage.

Cliff, Levene and Sanders (1963) studied collagen formation in the rabbit ear-chamber in relation to the effects produced by the administration of lathyrogenic compounds. The effects of BAPN and isonicotinic acid hydrazide (INAH) on wound healing, using the rabbit ear-chamber technique, were studied with the aid of electron microscopy. The results showed the presence of both fibroblasts and extracellular collagen fibrils to be distinguishable from those in the control group. They concluded that the formation of collagen was not inhibited by the lathyrogenic compounds used, which disagreed with earlier works that pointed to a diminution in collagen synthesis after lathyrogenic treatment (Enzinger and Warner, 1960).

Recent studies on the lathyrogenic syndrome have been reported by Tanzer and Gross (1964). This study was conducted using 14-day chick embryos and employing radioisotopic incorporation studies on normal and lathyritic embryos. The investigation reported the disturbance of two maturation processes:

(1) formation of intramolecular cross-links.

(2) decrease in fibril solubility.

It appeared that the lathyritic process affected collagen in all states of aggregation. The radioisotopic results showed that, in the embryonic collagen, there was no interference by lathyrogens with collagen synthesis or fibril formation.

Simmons, Pankovich and Budy (1965) investigated osteolathyrism by studying the effect of AAN on the skeletons of young mice injected with estrogen. Using a histological and microradiographical index, they found

that estrogen protected epiphyseal cartilage against degenerative changes after low doses of AAN, but the hormone was only partially effective after higher doses of AAN. This corroborated the study by Ponseti and Shepard (1954) which showed that estrogen appeared to inhibit the development of lathyrism in the rat. The results of the study by Simmons, Pankovich and Budy (1965) showed a diminution in bone ossification, a demineralization of cortical bone and the development of a mosaic appearance in the distal femur of the AAN-treated mice. There was little evidence to suggest that the functional activity of osteoblasts was impaired by AAN, but there was evidence which suggested that AAN disrupted both the formation of new bone and the integrity of collagenous structures deposited prior to treatment.

CHAPTER III

MATERIALS AND METHODS

The fertile eggs used in this study were obtained from the Georgia State Hatchery, Atlanta, Georgia. The eggs were incubated at 38.5° C. under controlled humidity, and turned twice a day. The inoculum was introduced by means of a hypodermic syringe and 25-gauge needle through a small opening in the air-sac end of the egg, according to the procedures outlined by Adams and Hirchinson (1959). The hole was sealed with soft paraffin and incubation continued at the original temperature. After the appropriate incubation time, the embryos were removed from the egg and placed in a 0.9% saline solution (NaCl) for observation. Each embryo was then fixed in an appropriate fixative. Sectioning of all tissue was made on a standard microtome, and sections were placed on clean albuminized slides and dried on a warming table to insure adhesion.

The lathyrogen employed in this study was propionitrile (Eastman Organic Chemicals, Rochester, New York). The concentrations of the injected lathyrogen (diluted with distilled water) were 1.0%, 0.1% and 0.01%. Injections of 0.5 cc of a particular concentration were made into the yolk after 48-hours of incubation. The injected eggs were sacrificed daily from 72- through 192-hours. Following gross observation, the embryos to be sectioned were placed in Bouin's fixative. After fixation (12 - 14 hours), they were washed in 70% ethyl alcohol, dehydrated, cleared, embedded by the paraffin method (Guyer, 1953), and sectioned at eight microns.

The slides were stained by Lison's Alcian blue-chlorantine fast red method. Humason (1962) described the method devised by Lison in which the Alcian blue method for acid mucopolysaccharides was counterstained with

chlorantine fast red for differentiation of mucin from collagen. The collagen fibers stain red and the mucin stains bluish-green. The staining procedure was to: (1) deparaffinize with xylol; (2) hydrate to water; (3) stain in Alcian blue for 30 minutes; (4) rinse in distilled water; (5) treat with 1% aqueous phosphomolybdic acid; (6) rinse in distilled water; (7) stain in chlorantine fast red for ten minutes; (8) dehydrate; (9) clear in xylol; (10) mount in balsam.

Two types of control embryos were used in this study:

(1) non-injected eggs

(2) eggs injected with 0.5 cc of distilled water.

The control embryos were incubated, sacrificed and stained in the same manner as the experimental eggs.

CHAPTER IV

EXPERIMENTAL RESULTS

The results obtained in this investigation were based on transverse sections of chick embryos (72- to 192-hours old). The sections were stained by Lison's Alcian blue-chlorantine fast red method in which collagen stained red, and cartilage and mucin bluish-green.

Two types of control embryos were used in this investigation: (1) non-injected eggs; (2) eggs injected with 0.5 cc of distilled water. Both types of controls were histologically normal. The primitive axis of the chick embryo (Fig. 1) consisted of a dorsal neural tube (NT) flanked on its sides by sclerotomal somites (S) which gave rise to mesenchymal cells (M). Ventral to the neural tube was the notochord (N), around which the future vertebrae formed. The vertebral column of the chick embryo developed from the mesenchymal cells of the sclerotomal somites. In the 72-hour chick embryo, the mesenchymal cells were connected with each other by a system of fine cellular processes which appeared to be a syncytium but may have been a group of cells in close apposition. According to Hamilton (1952), these mesenchymal cells and their processes migrated toward, extended around and became closely applied to the notochord, where the processes gave rise to the fibrous notochordal sheath (Fig. 2, NS).

Microscopic examination of the 4-day-old embryo showed an apparent increase in number and a spreading out of the migrated mesenchymal cells to form a continuous covering, the perichordal layer (Fig. 3, PL). This perichordal layer became intimately applied to the notochordal sheath. The 5-day embryo (Fig. 4) possessed spindle-shaped cells arranged concentrically around the notochord to form the centrum (C). The vertebra (Fig. 5) was

represented by the perichordal layer of loose mesenchyme (LM), the centrum and two mesenchymatous arches (MA) that ascended from the centrum to the sides of the neural tube. Some red-staining fibers were present in the centrum, indicating the presence of collagen. The 6-day-old embryo varied only slightly from the preceding stage; in this case, more red-staining fibers were present and the vertebrae appeared to increase in size by peripheral growth. By the seventh day of incubation the basic plan of the vertebra was established.

The neural tube developed dorsal to the notochord and consisted of epithelial cells, the inner-most of which became ciliated in the 3-day chick embryo. The tube, which increased in size from the fourth through eighth day, possessed three regions (Figs. 6 and 7): (1) the inner-most zone of mitotic epithelial cells or germinative cells (G); (2) the middle zone (MZ) of epithelial cells and nuclei; (3) the outer-zone or non-nucleated fibrillar margin (MV). As the germinative cells multiplied, the wall of the tube increased in thickness. The gray matter of the cord formed from the middle zone or mantle layer, and the white matter developed from the marginal velum. The neural canal was elongated up to the sixth day, after which the dorsal portion became obliterated by the lengthening of cells of the roof; the ventral division of the central canal remained as a permanent cavity.

A total of seventy-two experimental embryos were used in this study. Two dozen 48-hour chick embryos were injected with 0.5 cc of 1.0% propionitrile and two dozen 48-hour embryos were injected with 0.5 cc of 0.1% propionitrile. In both cases, the lathyrogen proved to be lethal after 24 hours.

Two dozen 48-hour embryos were injected with 0.5 cc of 0.01% propionitrile. Sixteen of these embryos survived and were sectioned and stained according to the procedure previously outlined. Observation of the external characteristics of some lathyritic embryos prior to fixation showed certain variations from the controls. Experimental embryos 3- through 6-days-old appeared normal; however, the 7-day and 8-day old embryos showed a decrease in body size. The outer covering of these embryos was thin and loose, and the entire organism appeared to have lost its rigidity.

Results of the histological study of the lathyritic embryos varied according to the age of the embryos. The developing vertebrae of the 3- to 6-day embryos were histologically normal when compared to the control groups. There was, however, a definite variation in the 7-day and 8-day embryos. The 7-day embryo possessed red-staining fibers in the region of the developing centrum, but only to a slight degree. Fiber formation which was clearly distinct in the control embryos was not evident in this experimental group.

A more noticeable variation was seen in the 8-day-old injected embryo. Figures 8 and 9 show the concentric pattern of the red "collagen" fibers (F) which were present in the 8-day-old control embryo. These fibers, located in the centrum of the developing vertebra, were more densely packed. Study of an injected embryo of this same age (Figs. 10 and 11) showed a dispersion of cells (DC) in the region of the vertebral centrum. The concentric pattern of red "collagen" fibers was greatly reduced, and only fibrillar-like processes (P) extended in a non-uniform pattern about the mesenchymal cells, leaving a space between the cells. The vertebral region stained differently

in these experimental embryos; there was a lack of red-staining material and the areas which were bluish-green in the control group appeared only as a dull hue.

The cells of the neural tube (Fig. 12) in the 8-day experimental embryo closely resembled the cells of the vertebra of the same embryo. The neural canal (NC) became occluded by dispersed cells.

CHAPTER V

DISCUSSION

The purpose of this investigation was to study the effects of a lathyrogenic agent, propionitrile, on the vertebral axis during embryogenesis. Studies on the chemical structures of lathyrogens by Levene (1961) showed that the lathyrogenic capacity of an organic nitrile was influenced by the presence of a reactive amine group. Propionitrile, one of the chemicals tested by Levene, produced lathyritic lesions and was therefore classified as a lathyrogen.

Although the embryological development of the vertebral column had been extensively investigated (Gadow, 1933; Baitsell, 1925; and Williams, 1942), this study led to a reinvestigation of the formation of the perichordal layer and its precise relationship to the formation of the vertebral centrum. The histological study of the developing vertebral column of normal chick embryos undertaken in this investigation supported the findings of Williams (1942) and Baitsell (1925).

The decrease in size of the chick embryo, noted in the 7-day and 8-day-old experimental animals, was found to be contrary to the results reported by Chang, Witschi and Ponseti (1955). Their investigation indicated no decrease in size when 14-day-old chick embryos were injected with the lathyrogen beta-aminopropionitrile, BAPN. The decrease in size of the chick embryo did, however, corroborate the works of Enzinger and Warner (1960), and Smith and Shuster (1962).

Although no histological study was made on the skin during this investigation, the loss of rigidity and the loose appearance which was observed in the 7-day and 8-day-old embryo, may be related to the breakdown of

dermal fibers (Van den Hooff, Levene and Gross, 1959; Kulonen, 1961). In these investigations, it was postulated that collagen fibers of the skin were affected by lathyrogenic agents. Van den Hooff, Levene and Gross (1959) investigated the skin of lathyritic chick embryos and found that dermal fibers were entirely absent in some areas, while only vestiges remained in other places. Enzinger and Warner (1960) reported a thinner appearance in the rat skin after injections of aminoacetonitrile, AAN.

Follis and Tousimis (1958) indicated that the developmental processes affected by lathyrogens were collagen fibers. The diminution of the normally concentric collagen fibers in the 7-day and 8-day-old experimental chick embryos indicated that there was a disruption of collagen fiber orientation. The histological method used in this study could not measure collagen synthesis, and there was no histological evidence that showed a decrease in the amount of collagen synthesized (using Lison's staining method). This apparent continued synthesis of collagen fibers agrees with the work of Enzinger and Warner (1960), Cliff, Levene and Sanders (1963), and Tanzer and Gross (1964). Tanzer and Gross used radioisotopic incorporation studies of 14-day-old chick embryos to investigate lathyrism and found that lathyrogens did not interfere with collagen synthesis or fibril formation.

The cartilage cells of the experimental embryos appeared dispersed in their position when compared to the control cells. This dispersion may be due to the non-uniform appearance of collagen fibers. Schryver and Biggers (1963) investigated the effects of lathyrogenic agents on embryonic chick tibiotarsus and attributed the dispersion of chondrocytes to a

dissolution of intercellular matrix. The clumping of cartilage cells and atypical proliferation which occurred in lathyritic rat bone (Milliser and Dasler, 1959) was not present in the chick embryo.

The change in staining color which was exhibited in the 8-day-old experimental embryo, gave an indication that the mucopolysaccharides, which normally stained bluish-green, may have stained a dull hue due to a change in chemical content. Gardner (1958) reported that there was some vital connection between the lathyrus factor and the chemical content of the mucopolysaccharides. Using the lathyritic rat skull, he concluded that lesions resulted from a degeneration of the mucopolysaccharides of the ground substance. Karnovsky and Karnovsky (1961) also concluded that lathyrogenic agents have a marked effect on the metabolism of the mucopolysaccharides of cartilage.

The dispersion of cells of the neural tube, which led to an occlusion of the neural canal, may also be a result of the degeneration of the ground substance as reported by Gardner (1958, 1960). In his works he stated that there was a possibility that lathyrogens affected the ground substance as well as the intercellular matrix.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. Histological techniques were employed to investigate the effects of propionitrile on the developing vertebrae of the chick embryo.
2. The vertebrae of the chick embryo developed from mesenchymal cells of the sclerotomal somites.
3. Collagen fibers were present in the developing vertebral centrum after 96-hours of incubation in the normal chick embryo.
4. The lathyrogen, propionitrile, in concentrations of 1.0% and 0.1% proved to be lethal to 2-day-old chick embryos 24 hours after injection.
5. Propionitrile of 0.01% concentration did not affect vertebral development of 3- through 6-day-old chick embryos, but did cause a decrease in size and loss of rigidity in 7- and 8-day-old chick embryos.
6. There was a dispersion of cells of the vertebrae in the 7- and 8-day-old experimental embryos, and fibrillar-like processes extended in a non-uniform pattern about the mesenchymal cells of the 8-day experimental embryo in which no concentric pattern of collagen fibers existed.
7. The chemical content of the mucopolysaccharides of the developing vertebrae was affected; this effect was indicated by the color variations of the mucin and cartilage which changed from the normal bluish-green to a dull hue.
8. The epithelial cells of the 8-day-old embryonic neural tube became dispersed in lathyritic chick embryos and occluded the neural canal.
9. Varying concentrations of the lathyrogen, propionitrile, have a definite effect on the developing vertebral axis of the chick embryo.

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PLATE I

(Explanation of Figures)

(Explanation of Figures)

Fig. 1. Photomicrograph of a transverse section through a normal 3-day-old chick embryo to show: neural tube (NT), sclerotomal somites (S), mesenchymal cells (M), and notochord (N). Alcian blue-chlorantine fast red. X450.

Fig. 2. Photomicrograph of a transverse section through a normal 3-day-old chick embryo. Note: fibrous notochordal sheath (NS), neural tube (NT), and notochord (N). Alcian blue-chlorantine fast red. X970.

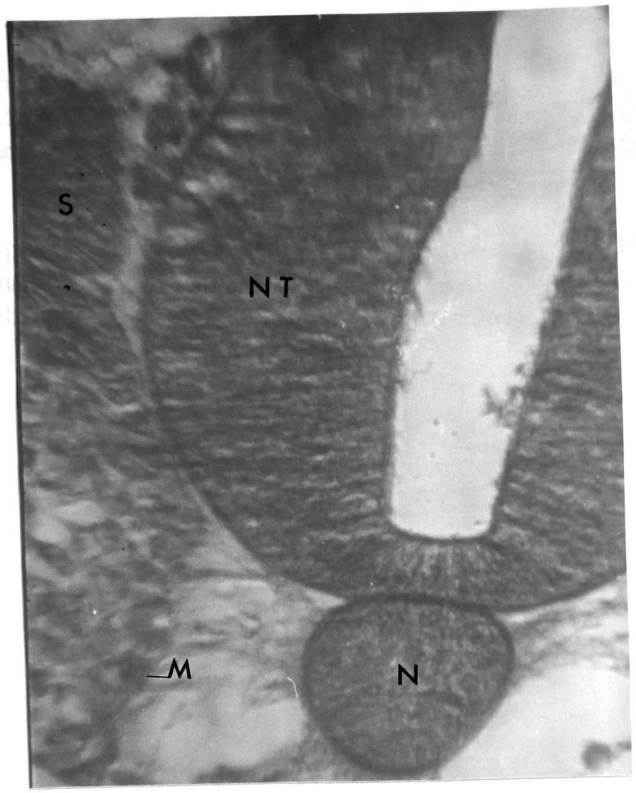

Fig. 1

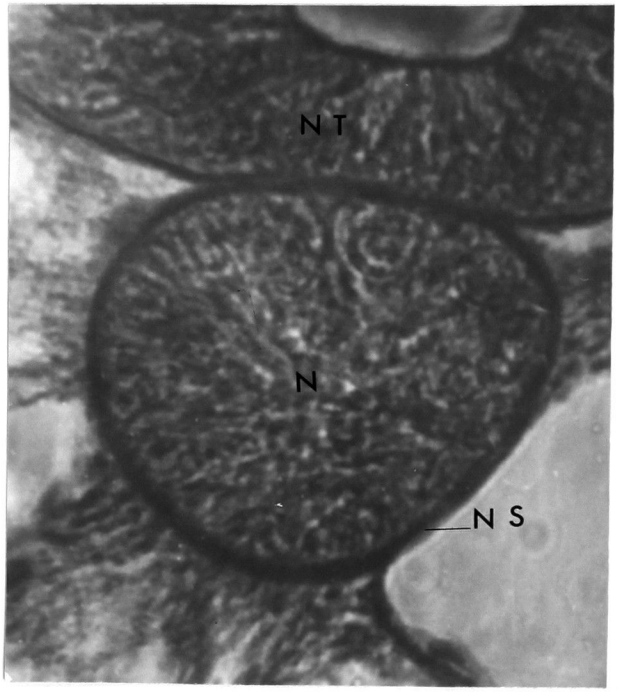

Fig. 2

(Explanation of Figures)

Fig. 3. Photomicrograph of a transverse section through the vertebral region of a 4-day chick embryo to show: perichordal layer (PL), neural tube (NT), and notochord (N). Alcian blue-chlorantine fast red. X970.

Fig. 4. Photomicrograph of a transverse section of a 5-day normal chick embryo to show: centrum (C), neural tube (NT), and notochord (N). Alcian blue-chlorantine fast red. X450.

PLATE II

(Explanation of Figures)

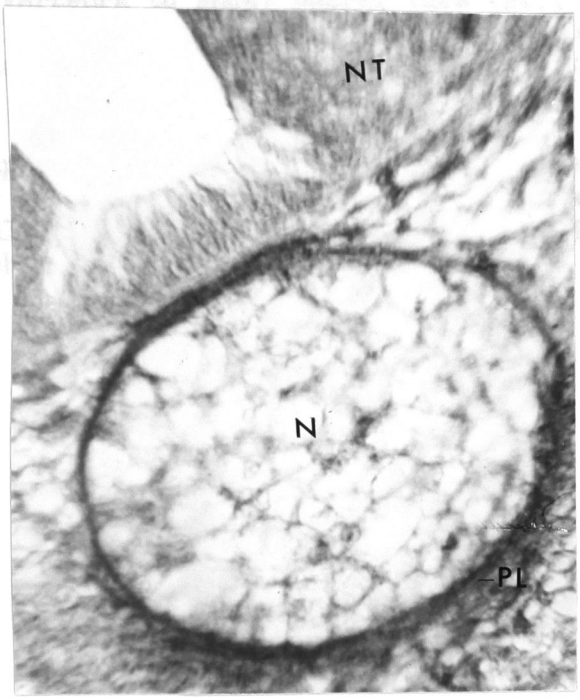

Fig. 3

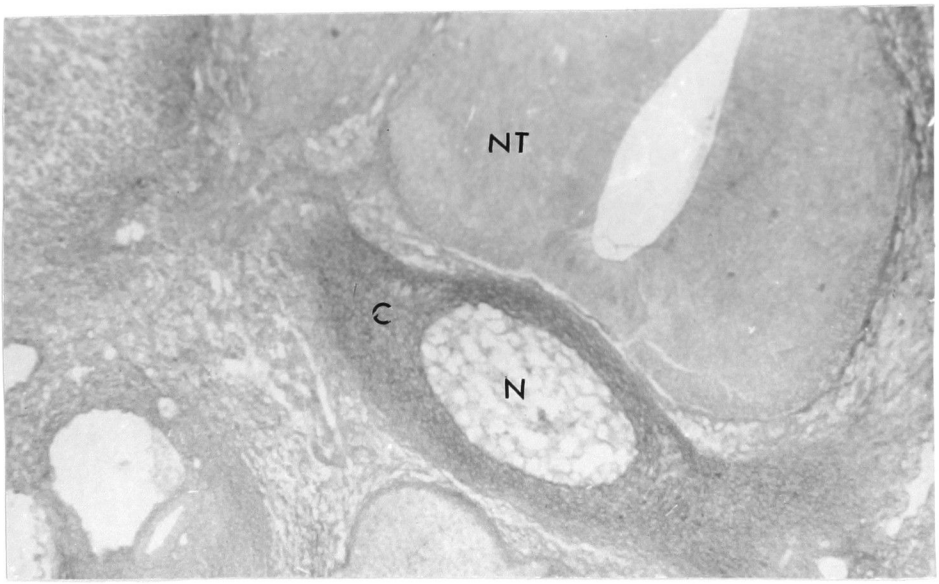

Fig. 4

(Explanation of Figures)

Fig. 5. Photomicrograph of a normal 5-day chick embryo through a region of a developing vertebra to show: neural tube (NT), notochord (N), centrum (C), loose mesenchyme (LM) of the perichordal layer, and mesenchymatous arches (MA). Alcian blue-chlorantine fast red. X450.

Fig. 6. Photomicrograph of a transverse section through a normal chick embryo showing: neural tube (NT), germinative cells (G), middle zone (MZ), marginal velum (MV), neural canal (NC), and notochord (N). Alcian blue-chlorantine-fast red. X450.

PLATE III

(Explanation of Figures)

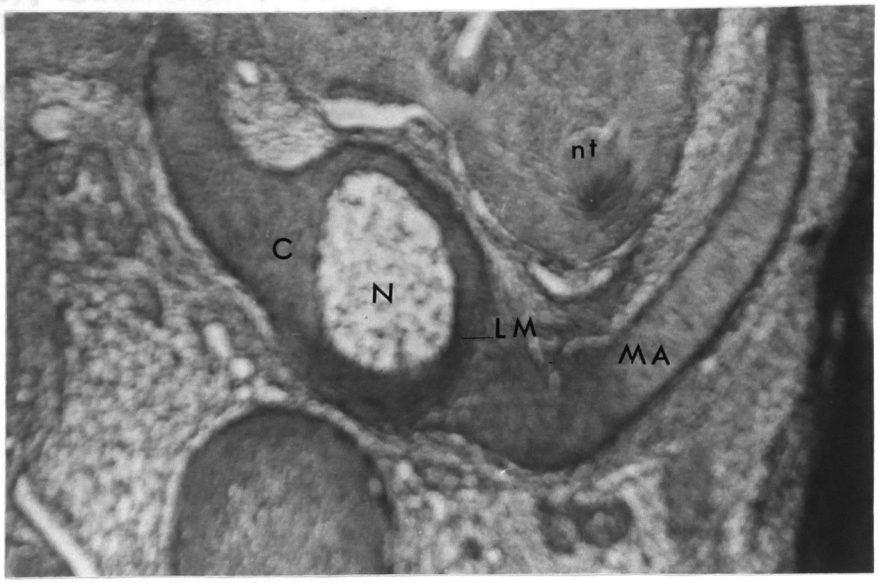

Fig. 5

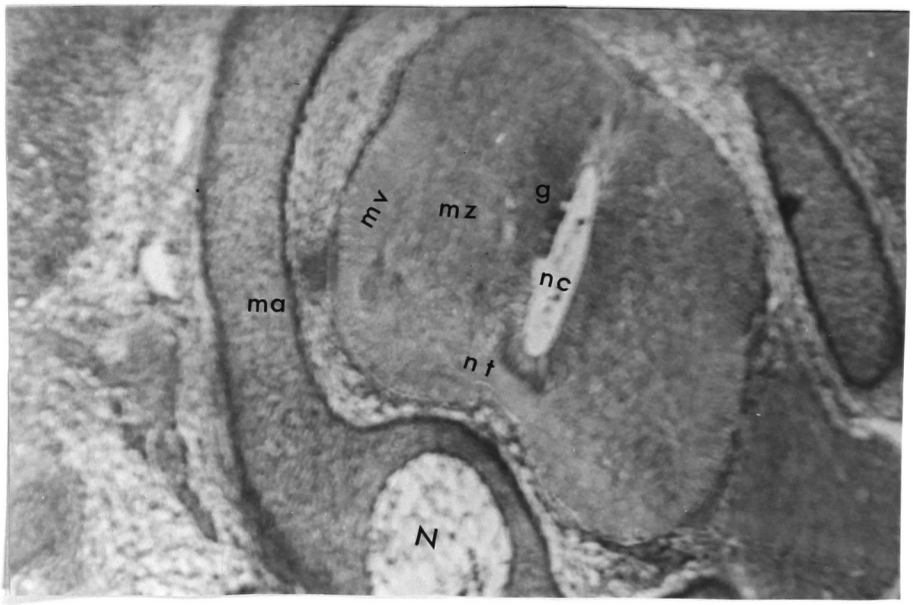

Fig. 6

(Explanation of Figures)

Fig. 7. Photomicrograph of a transverse section through a portion of the neural tube of a normal 5-day chick embryo to show: germinative cells (G), middle zone (MZ), and marginal velum (MV). Alcian blue-chlorantine fast red. X970.

Fig. 8. Photomicrograph of a transverse section through a developing vertebra of an 8-day control chick embryo showing: notochord (N), and "collagen" fibers (F) in the developing centrum. Alcian blue-chlorantine fast red. X450.

PLATE IV

(Explanation of Figures)

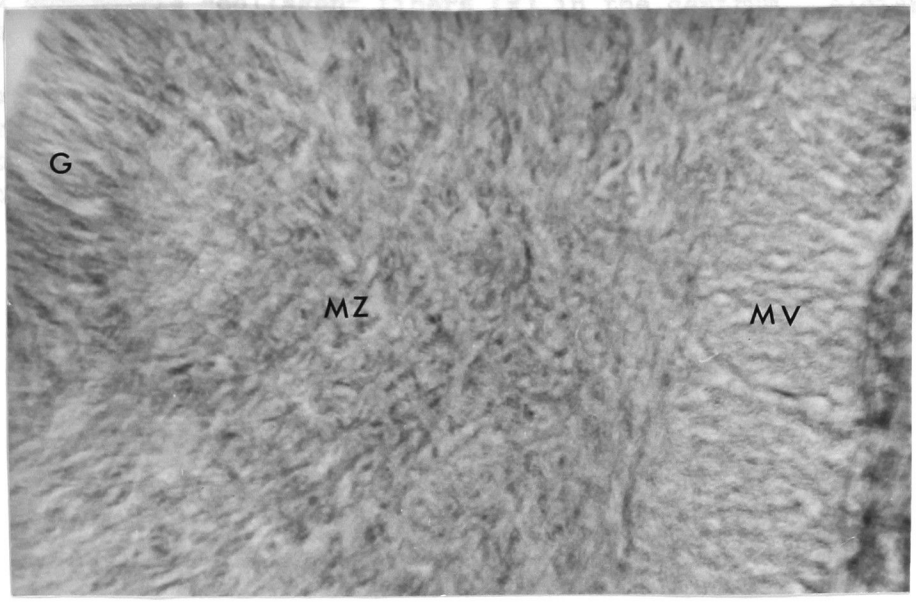

Fig. 7

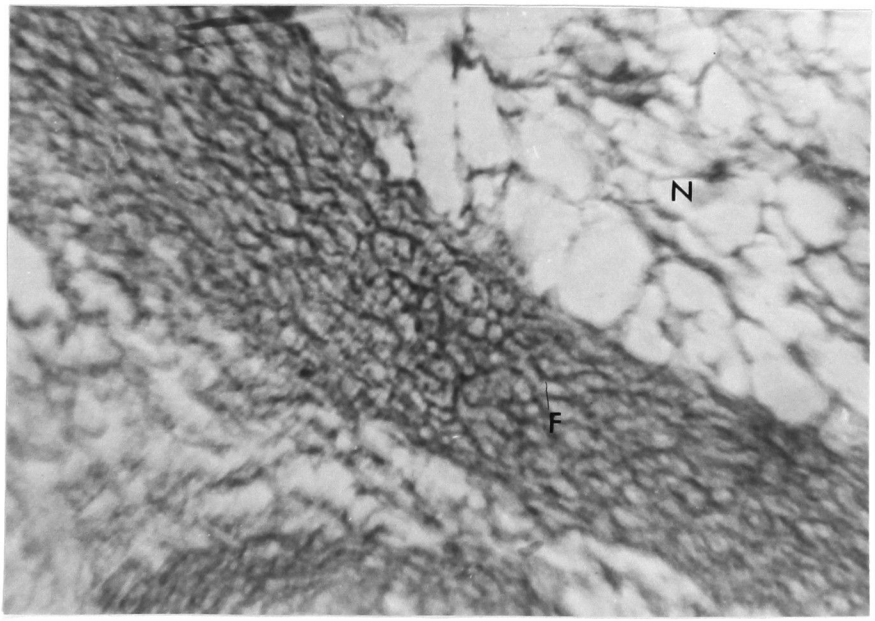

Fig. 8

(Explanation of Figures)

Fig. 9. Photomicrograph of a transverse section through the developing vertebra of an 8-day control chick embryo showing: notochord (N), and concentric "collagen" fibers (F) in the centrum. Alcian blue-chlorantine fast red. X970.

Fig. 10. Photomicrograph of a transverse section through the region of a developing vertebra in an 8-day experimental chick embryo. Note: notochord (N), neural tube (NT), and dispersed cells (DC). Alcian blue-chlorantine fast red. X 450.

PLATE V

(Explanation of Figures)

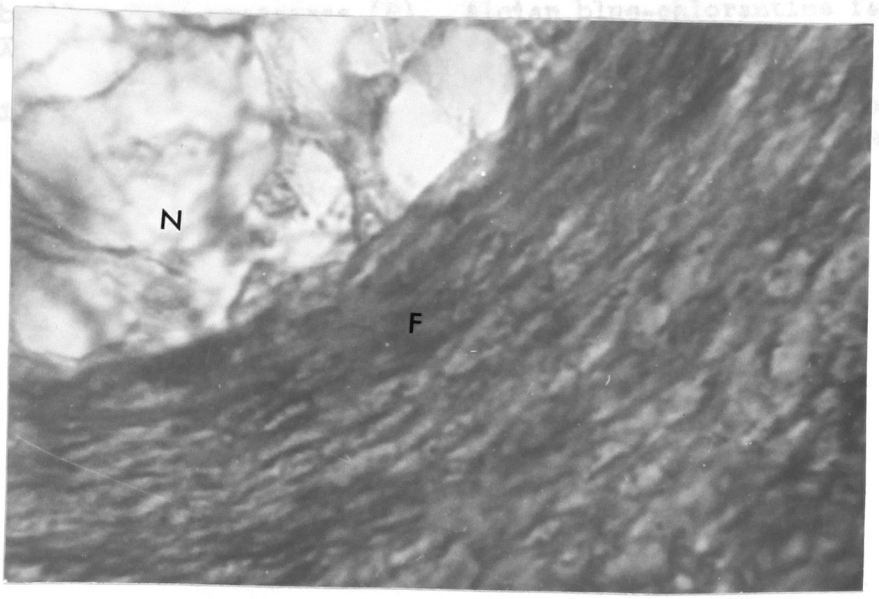

Fig. 9

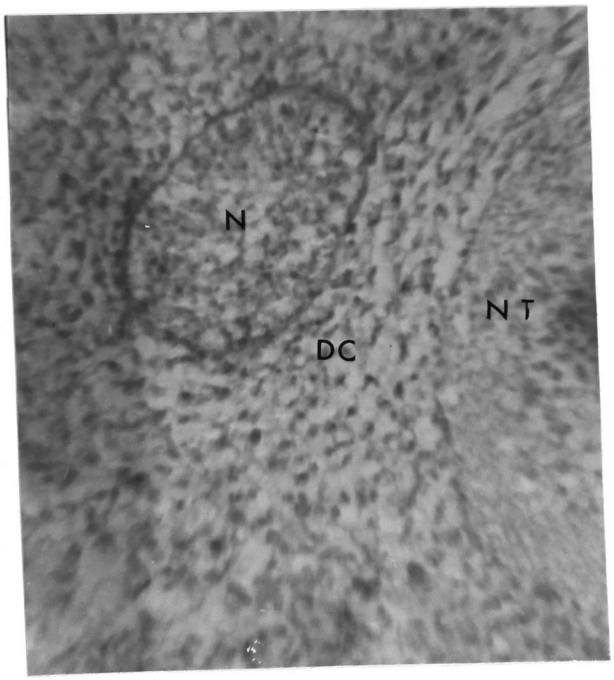

Fig. 10

(Explanation of Figures)

Fig. 11. Photomicrograph of a transverse section through an 8-day experimental chick embryo. Note: notochord (N), dispersed cells (DC), and fibrillar-like processes (P). Alcian blue-chlorantine fast red. X970.

Fig. 12. Photomicrograph of a transverse section through an 8-day experimental chick embryo showing: neural tube (NT), and neural canal (NC). Alcian blue-chlorantine fast red. X970.

PLATE VI

(Explanation of Figures)

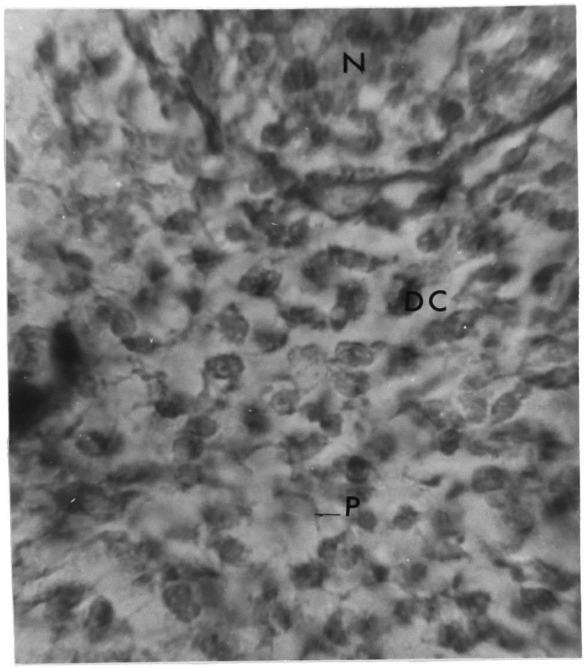

Fig. 11

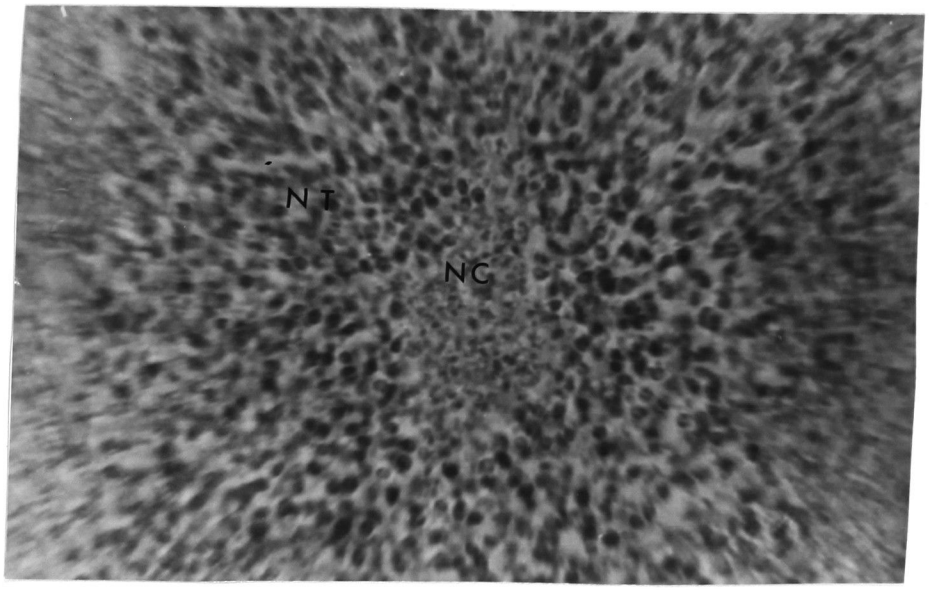

Fig. 12